Appl. No.

: 10/009,792

Filed

December 13, 2001

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

- 1. (Currently Amended) A recombinant plasmid vector which comprises:
- a kanamycin resistance gene;
- a promoter;
- a nucleotide sequence coding for an endoxylanase signal sequence;
- a nucleotide sequence coding for an oligopeptide consisting of 13 amino acids including 6 consecutive histidine residues; and,
 - a human granulocyte colony stimulating factor (hG-CSF) gene.
- 2. (Currently amended) The recombinant plasmid vector of claim 1, wherein the nucleotide sequence codes for an oligopeptide has an which comprises an amino acid sequence of isoleucine-glutamic acid-glycine-arginine (Ile-Glu-Gly-Arg; SEQ ID NO: 28) within the oligopeptide.
- 3. (Currently amended) A recombinant plasmid vector, pTHKCSFmII represented in Figure 13 which comprises:
 - a kanamycin resistance gene;
 - a Trc promoter;
- <u>a nucleotide sequence coding for</u> an endoxylanase signal sequence derived from *Bacillus* sp.;
- a nucleotide sequence coding for an the oligopeptide of SEQ ID NO: 1, which contains six consecutive histidine residues in the sequence AGPHHHHHHH and the protease target sequence IEGR; and
- a modified gene coding for a human granulocyte colony stimulating factor (hG-CSF); which includes a nucleotide sequence of SEQ ID NO: 26 at the N-terminus.

 wherein the nucleotide sequences coding for the endoxylanase signal sequence, the oligopeptide of SEQ ID NO: 1 and N-terminal portion of the mature hG-CSF are present in SEQ ID NO: 26.

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4. (Original) A microorganism, *E. coli* transformed with the plasmid vector, pTHKCSFmII of claim 3.

- 5. (Original) The microorganism of claim 4, wherein the *E. coli* is selected from the group consisting of *E. coli* XL1-Blue, *E. coli* MC4100, *E. coli* BL21 (DE3), *E. coli* HB101 and *E. coli* W3110.
- 6. (Original) E. coli MC4100/pTHKCSFmII (KCTC 0754BP) transformed with the plasmid vector, pTHKCSFmII of claim 3.
- 7. (Original) A process for preparing a human granulocyte colony stimulating factor, which comprises the steps of:

culturing *E. coli* transformed with the plasmid vector of claim 1 to obtain a human granulocyte colony stimulating factor fusion protein; and,

treating the human granulocyte colony stimulating factor fusion protein with a protease to obtain a human granulocyte colony stimulating factor.

- 8. (Original) The process for preparing a human granulocyte colony stimulating factor of claim 7, wherein the plasmid vector of claim 1 is pTHKCSFmII.
- 9. (Original) The process for preparing a human granulocyte colony stimulating factor of claim 7, wherein the human granulocyte colony stimulating factor fusion protein is obtained from the culture by employing Ni-column.
- 10. (Original) The process for preparing a human granulocyte colony stimulating factor of claim 7, wherein the protease is Factor Xa.